INAUGURAL
SCIENTIFIC MEETING
JUNE 21-22, 2021

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Conference Summary Report

compiled by:

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TABLE OF CONTENTS

Glut1 Deficiency Foundation Welcome and Goals:

Jason Meyers, Sandra Ojeda, Glenna Steele

The Glut1 Deficiency Foundation is a nonprofit patient advocacy organization dedicated to improving lives in the Glut1 Deficiency community through its mission of increased awareness, improved education, advocacy for patients and families, and support and funding for research.

This inaugural scientific meeting is part of an effort to establish a patient-led collaborative research network and create a strategic roadmap to drive progress towards better understanding, better treatments, and one day a cure.

The goals of this meeting:

- Share key areas of current research in Glut1 Deficiency and identify critical gaps
- Establish connections and identify overlaps and synergies among related research areas
- Identify potential researchers for participation in a collaborative research network
- Identify potential projects for strategic research plan to drive progress

Dr. Matthew Gentry, the G1DF Science Advisor, has been instrumental in planning this meeting, expanding the way we think about this disease, and helping turn our hopes and dreams into actions.

Glut 1 Deficiency Clinical Insights:

Glut1 Deficiency Syndrome 1991-2021: Lessons Learned from a Quintessential Rare Disease

Darryl De Vivo, MD

The highlights as we have come to understand G1DF can be divided into 3 decades:

1st decade:

- Recognize and introduce this rare disease to professionals and the public
- Develop some form of treatment
- Understand the molecular basis of the disease

While the first two cases were in 1991, and it was suspected that there was a defect in the transport of glucose from the blood into the brain, it wasn't until 1998 that it was reported that a mutation in the SLC2A1 gene caused this condition.

The ketogenic diet was introduced in 1991 as the standard of care. Although glucose is the primary fuel for brain metabolism, ketone bodies are alternative fuel to meet the needs of the developing brain.

2nd decade:

- Pathophysiology of the condition
- Developed a mouse model which was published in 2006 that captured well the condition

3rd decade:

- Effort to develop more effective disease modifying treatment, particularly gene therapy
- Realized there was a disturbance in the development of cerebral microvasculature
- The point prevalence of the disease was increasing

Original report in the NEJM in 1991: 2 infants presenting with seizures, developmental delay, decelerated head growth and movement disorder. This has become the classical phenotype in 80-90% of patients.

2 key biomarkers:

- low CSF glucose

- low CSF lactate

Introduced at that time the ketogenic diet therapy (KDT) as the standard of care. Early diagnosis facilitates early treatment. A delay between initial presentation and correct diagnosis creates missed opportunity to treat the patient early with KDT.

The course of the disease in first patient diagnosed with Glut1 Deficiency:

- myoclonic seizures in infancy
- movement disorder in adulthood
- moderate intellectual disability
- neurobehavioral disturbances

This profile has characterized the majority of Glut1 Deficiency patients.

The neurological domains affected in Glut1 Deficiency:

- *Cognitive phenotype* domain which is a lifelong disability
- *Epileptic phenotype* which includes migraines and disturbances in behavior
- *Dyskinetic phenotype* that emerges as the epileptic phenotype resolves and is characterized by a number of movement disorders such as spasticity, ataxia, and dystonia

The management of the patients has remained constant over the past 30 years.

- Early diagnosis and treatment improves outcome
- KDT remains as the standard of care
- It is recommended to measure ketone bodies in the blood as opposed to the urine
- A multidisciplinary approach is important (physicians, therapists, dieticians, etc.)

The cessation of symptoms with rise in glucose and insulin suggests that if able to maintain an elevated blood glucose, we can improve these patient's symptoms.

The mouse model has revealed that restoring Glut1 early in life, pre-symptomatically, protects against Glut1 Deficiency. However, if restored later in symptomatic mice, it fails to rescue phenotype.

Newborn screening test is needed to be able to determine potential patients within therapeutic window.

Symptoms and Biomarkers

Dr. Juan Pascual

The main emphasis will be on biomarkers which is something that you can measure that can tell you how your body may be doing. However, a problem with biomarkers is that they do not necessarily correlate with what a person is experiencing. We should be moving towards things that are meaningful for the patient rather than a laboratory assay.

Some biomarkers used for Glut1 Deficiency and the challenges:

DNA:

- High negative rate - some areas of the gene are not conventionally tested
- There could also be another gene that causes the disorder
- There is a great deal of uncertainty in DNA
- Mutation severity does not necessarily correlate with disorder severity
- The type of mutation may or may not predict the evolution of the person

EEG:

- Dependent on multiple factors including the time of day and time that you had a meal
- EEG can't really be used to quantify how a person may be doing
- Improvements in an EEG does not necessarily improve cognitive performance of patient which is what matters most to a person

CSF glucose and lactate:

- Unclear how low you need to be to be diagnosed
- Can be normal in some people
- It is not necessarily the case that someone with lower level is doing worse

Red blood cell uptake:

- Drawback is that it involves radioactivity
- Red blood cells tend to lose glucose uptake over time
- It is mutation dependent in order to be informative

Glut 1 transport kinetics:

- There are many components to the process of Glut1 transport

PET:

- Difficult to qualify how much glucose signal there is in the brain

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Natural History

Prof. Dr. Michel Willemsen

Signs and Symptoms:

- Intellectual disability – generally stable throughout the lifespan
- Epilepsy – tends to decrease in severity and in frequency of seizures during late childhood and adolescence
- Movement disorder – tends to increase during adolescence
- Good response to KDT makes the disorder recognizable
- Microcephaly – ½ to 2/3 of patients have microcephaly
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Almost all papers include microcephaly as a clinical characteristic of Glut1 Deficiency. There is a close relationship between the development of brain and head size; because of this, neurologists often study head size in disorders. This is important because Glut1 Deficiency is not only an error of metabolism but also results in abnormalities in the microvasculature of the brain - defect in brain vessels.

In an effort to determine whether microcephaly is really common in Glut1 Deficiency patients, a retrospective study collecting head circumference was done. It was found that microcephaly is not as common as usually thought and if it occurs, it's mild. Glut1 Deficiency patients had a normal development of head growth. Almost all children had a head circumference range from -2 to 2, which is in normal range. They tend to have lower than average but not at an abnormal measurement.

Current Treatment Options- What Works, What Doesn't, What Might

Prof. Dr. Jörg Klepper

Current treatment options:

Ketogenic Dietary Therapy:
Treatment of choice because with Glut1 Deficiency there is an energy crisis and we can refuel the brain with ketones instead of glucose. Has been shown to be most helpful in young children with epilepsy and does positively influence movement disorders. However, in older children and adults the KDT doesn't seem to help much with other movement disorders (such as paroxysmal dystonia) or speech difficulties.

KDT ratio recommendations
4:1 – can be done in children, should not be used in infants
3:1 – the most recommended diet in children and infants up to 2 years. With Glut1 Deficiency, you should try to maintain as high a ketogenic diet as you possibly can.

Modified Atkins Diet is preferred by most parents because it's easier to apply but generates lower levels of ketosis. It is more commonly applied in adolescents and adults.

Low glycemic Index Diet is not recommended for Glut1 Deficiency.

Oral ketones and ketoesters:
May serve as a supplemental fuel for the brain. Ketone salts can achieve adequate ketosis, however, you need huge volumes to achieve ketosis and may cause sodium overload.

Anti-epileptic drugs (AED):
There are children that continue to suffer from epilepsy despite adequate ketosis with KDT. Unfortunately, anti-epileptics tend to be ineffective for this condition. There is very little data on the interaction of AED and KDT in Glut1 Deficiency and no drug can be recommended at the moment.

Movement disorder drugs:
Triheptanoin: did not reduce seizures or movement events. Could be possible that another combination of KDT and triheptanoin ratio is needed and needs to be explored.

Acetazolamide:
Older drug that has been used against epilepsy for decades. In some Glut1 Deficiency patients, it helped with movement abnormalities.

Levodopa: In one older patient, it helped with paroxysmal exercise-induced dystonia

Small molecules:
How signaling pathways will help to fuel the engine of producing energy which helps with transporting glucose in Glut1.

Gene therapy:
Essentially, you have a virus that gets filled with a vector and this vector is a healthy Glut1 gene. The virus then infects a Glut1 deficient mouse and this gene produces healthy Glut1 molecules, curing the condition. This has been proven to be effective in mice but there is still a lot of work to do before this can be applied to humans. It will take some time to be able to prove it is safe and effective in humans but it seems it may be very effective.

Glut1 Deficiency Current Research Session 1:

Delineating the Spatial and Temporal Requirements for Glut1 in Glut1 Deficiency

Umrao Monani, PhD

In mice where Glut1 Deficiency was induced, motor performance was affected in mutants, EEG activity disrupted, and brain size reduced.

Reduced brain microvasculature is caused by endothelial tip cell defects and in the mouse model, it was found that in Glut1 Deficiency, there are fewer endothelial tip cells. Massive neuroinflammation in astrocytes and microglia was also found, which was accompanied by prominent cell loss.

Conclusion regarding Glut1 spatial requirements:

- Glut1 is important for brain endothelial cells- making these cells haploinsufficient for Glut1 is sufficient to induce all the signature feature of Glut1 Deficiency
- Defects in brain angiogenesis is triggered in a cell autonomous fashion
- Low Glut1 in endothelial cells can trigger other brain pathologies such as neuroinflammation and neuronal loss
- Targeting brain endothelial cells will be imperative in any future Glut1 repletion therapies

Defining the temporal requirements for Glut1:

Inducing Glut1 Deficiency at postnatal day 2 resulted in classic Glut1 Deficiency phenotype as measured by Glut1 transcripts, proteins, motor performance, seizure-like activity, and reduced endothelial tip cells.

Inducing Glut1 Deficiency at later stages has milder effects. There is a 50% or more reduction of Glut1 transcripts at later stages, but in motor impairment and brain size the impairment wasn't as great in animals induced at adulthood compared to juvenile stages.

Glut1 mutations act through endothelial tip cells (critical for brain angiogenesis) and failure to target these cells in future treatment is unlikely to provide optimal benefit.

Gene Therapy for Glut1 Deficiency

Hitoshi Osaka, MD, PhD

Tested the function of Glut1 AAV vector variation for the cell. Tested various injection routes (IP, ICV, and best expression was ICV followed by IP).

It was found that after injection, Glut1 mRNA expression increased and brain microvasculature as well as motor function was improved. CSF glucose levels were also improved and it was confirmed that increased expression was maintained for 24 months.

The injection has been tested in a pig with injection via catheter and broad CNS delivery of vector was obtained and high levels of Glut1 mRNA were obtained in the brain.

There are plans to start a Phase I/IIa clinical trial in 2022.
Inclusion criteria for Japanese clinical trial: older than 2 years of age
The plan is to start with older patients who are not seeing improvement with KDT and then transition to younger patients.

RNA Therapy Approach for Glut1 Deficiency using SINEUPs

Federico Zara, MD

Rationale for this project:
Disease is caused by haploinsufficiency of Glut1. There is significant correlation between clinical severity and the degree of impairment of glucose uptake. Glut1 transporter is a molecular bottleneck that is located in brain microcapillary endothelial cells. In human brain endothelial cells, glucose uptake is specifically carried out by Glut1 transporter.

There are novel tools that can be used to treat haploinsufficiency. SINEUPs are a new class of antisense long non-coding RNAs that up-regulate translation of target proteins. SINEUPs can be constructed to target the genes that we want to target. The mRNA is not altered in SINEUPs but they upregulate proteins to rescue the phenotype.

We tested whether SINEUPs are able to increase translation of the Glut1 protein and it was found that at 24 hr and 48 hr, these SINEUPs do not result in a significant increase in expression. However, transfection efficacy tests and more replicates are needed.

The next task was to test the implementation of an in vitro BBB model using human GLUT1 deficient brain endothelial cells and found that in patient cell lines, there was decreased glucose uptake.

These are very preliminary results and next steps are:

- Design of additional SINEUPs
- Optimization of Glut1 quantification to detect small effects

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Novel Genetic Interactions

Paolo Grumati, PhD

This laboratory focuses on autophagy which is the cellular cleaning system.
Autophagy:

- eliminates cytosolic material via lysosomes
- maintains the cellular energy balance during fasting and stress
- regulates the physiological turnover of the cellular organelles
- degrades toxic protein aggregates and harmful organelles

The molecular mechanism that regulates autophagosome formation and lysosomal delivery are very well studied and characterized. Autophagy proteins also regulate cytosolic membrane trafficking. Autophagy controls Glut1 translocation to the plasma membrane.

Working to understand the autophagy machinery may be helpful in the development of a therapeutic target because it may positively affect the translocation of Glut1 from the endosome to the plasma membrane.

Ongoing studies:

- mass spectrometry analysis modulating autophagy flux
- identify the autophagy protein involved in glut1 translocation
- pharmacologically improve Glut1 translocation

Learning from Other Rare Genetic Diseases and the Non-Coding Genome

T.S. Stefan Barakat, MD, PhD

This lab is interested in the genetics of neurogenetic disorders - in particular, the non-coding genome. We are interested in understanding missing heritability and the role of the non-coding genome in neurogenetic disorders. Two main focuses:

- Can identification of regulatory elements underlying SLC2A1 regulation help explain missing heritability and offer future targets for therapy?
- Can modulation of nucleotide glucose metabolism help find a therapy for Glut1 Deficiency?

Missing heritability remains a problem in clinical genetics - oftentimes there is a high suspicion of a genetic disorder but unable to properly identify it. Causes of this could be non-coding genetic alterations. Targets for this are enhancers which are important regulators within the non-coding genome.

Alterations of enhancers are known to cause disease but enhancers are not routinely analyzed because the genetic search base to identify enhancers is very large. This laboratory developed a technology to shrink the searchbase for enhancers called ChIP-STARR-seq. It allows you to identify enhancer sequences, genome wide by only looking at the 5-10% of genome that really shows enhancer activity which makes it much easier to identify genetic variants that might cause disease.

We hope to shed more light into the regulatory elements underlying SLC2A1 regulation, particularly in patients that present with Glut1 Deficiency but no mutation is detected.

Interested in collaborating to:

- screen regulatory elements for variants that might cause Glut1 DS
- explore the alterations of nucleotide sugar metabolism

Glut 1 Deficiency Current Research Strategy Session 2:

Identifying New Treatments for Glut1 Deficiency

Jason Park, MD, PhD

As we know, there are limited treatment options for Glut1 Deficiency.
The major treatment options are dietary treatments such as the ketogenic diet and triheptanoin.

Multiple Glut1 research programs in other diseases have identified activators and inhibitors:
Examples:

Alzheimer's Disease → Metformin (activator)
Diabetic Neuropathy → Rosiglitazone (activator)
Cancer → Glut1 inhibitors

How can we quickly identify Glut1 activating compounds with high throughput screening? The UT Southwestern cancer center uses an assay system to refine and identify potential compounds. This is important because the model for finding Glut1 activators is lung cancer cells because they have high Glut1 expression. Additionally, the other glucose transporters (2-14) are low in these cells.

The main goal is to repurpose existing drugs to new diseases, trying to identify Glut1 activators from previously studied drugs. Successfully screened drug libraries and 120 Glut1 activators have been identified from over 10,000 compounds. The majority of these have existing preclinical data.

We have started characterizing a number of these compounds in mouse models and activating compounds are being applied to the Glut1 Deficiency mouse model.

Precision Medicine

Prof. Dr. Matthias Selbach

Focus is on the dynamic proteome to better understand how the gene affects the phenotype. We used a peptide-based interaction screen with disease causing mutation.

Findings:

- Not all pathogenic mutations lead to dysfunctional proteins - some cause protein mistrafficking
- A mutation in a disordered cytosolic tail of Glut1 creates a dileucine motif and causes clathrin-dependent endocytosis and trafficking of Glut1
- Preventing endocytosis restores Glut1 levels in the plasma membrane and glucose transport
- Mutation in disordered regions can cause disease by creating dileucine motifs

Ongoing studies: test approved drugs to increase levels of Glut1 in the plasma membrane

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New Ways to Think of Glut1 Deficiency

Juan Pascual, MD, PhD

Obstacles: the current treatments that are out there for Glut1 Deficiency each have their limitations

Metabolism – excitability in relation to Glut1
Metabolism has a catabolic and anabolic component, both of which are equally important in metabolism. This is emphasized by the large quantity of glucose that the child brain consumes, which is a lot less in adults. Metabolism supports neuronal excitability, you can't have one without the other. Decreased metabolism causes the increased excitability that leads to seizures.

Glucose leads to fundamental neurochemicals through the CREB cycle: Glutamate, Glutamine, and GABA are direct byproducts of glucose.

In the Glut1 mouse model, they experience seizures about every 10 minutes or so and what's happening is that both within the cortex and thalamus the magnitude of inhibitory synaptic events which are driven by GABA is very low. They don’t release GABA the way they are supposed to be releasing it.

With that in mind, the molecule being worked on - triheptanoin - is composed of glycerol and 3 of the heptanoic acids. It works to fuel both catabolism and anabolism. KDT fuels catabolism but heptanoate fuels both catabolism and anabolism.

The vast majority of drugs inhibit because it is easier to inhibit/ block/ decrease something as opposed to augment it. Inhibition usually leads to collateral effects which mandates that the mechanism be well understood to be able to understand the side effects. Metabolic therapies usually augment because there are fewer side effects from the stimulation of natural processes.

Current clinical trial on triheptanoate:
The patients were treated acutely for 6 months and then stopped for 3 months. It seems to be that this treatment leads to an increase in gamma activity and connectivity across different brain regions has increased in a number of people and in many cases that correlates with cognitive outcome.

Mass action treatment of Glut1 Deficiency:
Human red blood cells are rich in Glut1 and transfer glucose to the brain capillary cells with little intervening plasma. Ask what would happen if you replace 80% blood with donor blood who are not Glut1 deficient. This has been tried in 3 subjects and this has nearly normalized the amount of Glut1 molecule in red blood cells as well as led to improvements in other areas. However, over time, this effect is lost because of cell turnover.

Groundwork for new model:
Current work is being done to create a pig model.

Patient and Family Perspectives:

Glut1 Deficiency Collective Voices Project
Glenna Steele and Sandra Ojeda, PhD

The goals of this project were to:

- Capture family stories and translate into data
- Better understand patient and family experiences
- Better define the range of symptoms
- Identify gaps in treatment and patient care
- Identify gaps in knowledge and understanding of this disease

230 responses were received from 33 countries. We will provide a snapshot of preliminary data that has not yet been cleaned or analyzed in depth – those more formal reports will come.

Diagnosis:

- achieving diagnosis is still a significant challenge, many visit 8 physicians before a correct Glut1 Deficiency diagnosis
- average age of diagnosis is 4.2 years

Genetic Testing:

- 92% have undergone genetic testing; 82% of these patients have reported a mutation found

Symptoms:

- first symptoms are typically unusual eye-head movements and seizures
- stamina/endurance and low energy have not traditionally been included in the classical phenotype, but a large number of patients report experiencing these symptoms

Seizures:

- 84% of patients have experienced seizures
- 2/3 no longer experience seizure; this may be as a result of the diet

Movements:

- 77% report muscle or movement issues
- ataxia is the most common movement disorder reported

Top symptoms negatively impacting quality of life:

- cognitive and intellectual difficulties
- speech/communication issues
- lack of independence

Puberty:

- 41% have experienced or are currently in puberty which is accompanied by changes in their symptoms and treatment effectiveness
- many reported worsening of movements and stamina/energy

Adulthood:

- 25% of patient responses are in adulthood
- many report improvements in seizure
- they report their quality of life overall as staying the same or improved over childhood
- 57% of adults are on KDT

Development:

- most developmental milestones are met but delayed in childhood, not all adult milestones met

Speech & Language:

- 85% are able to communicate using their own voice
- half feel that their speech issues make them appear to be less capable/intelligent than they actually are

Cognitive:

- 74% receive special education services
- only 56% reported neuropsychological testing
- only 25% knew their IQ scores which is an important measure used for determination of services
- majority of scores reported fall within the 60-70 range

Social & Emotional

- patients report speech articulation, cognitive or intellectual difficulties and the social challenges around the KDT as the top 3 challenges that impact their social life

Ketogenic Diets:

- 91% have tried KDT
- some report diminished benefit from the diet over time
- KDT benefits: primarily seizure and movement disorder improvement
- KDT challenges: feeling different, impact on family celebrations and holidays, lack of opportunities for spontaneity
- Most families report that the benefits outweigh the challenges

Other treatments:

- 35% of patients reported having tried seizure medications, with keppra being the most common
- 14% have tried C7 oil / triheptanoin

Top 3 research priorities were:

- new and better treatments
- basic science for better understanding of the disease
- changes in adulthood

Top 3 priority outcomes for new treatments were:

- ability to eat a normal diet
- improved cognition
- better speech/communication

Many patients reported being open to participating in clinical trials

Glut1 Structure, Function, Regulation:

Glut1 Structure and Function

Prof. Tatiana Galochkina

Glut1 is responsible for glucose uptake in erythrocytes and endothelial cells in the blood brain barrier and any disruption of this results in dysfunction in the nervous system.

Increased expression levels of Glut1 are observed in malignant cells.

Glut1 is the most rigorously characterized glucose transport yet there are still many details of its mechanism of function which remain unclear.

The question being addressed is how exactly Glut1 mediates glucose transport at the molecular level.

Glut1 3D structure: it is an alpha protein and it makes part of the major facilitator superfamily (MFS) proteins which all share the following characteristics:

- 12 transmembrane helices
- 2 domains
- Huge intracellular loop

Glut1 must be able to adapt to different states – the state allowing glucose to enter, to move through, and to be able to exit.

The purpose of the studies is to reproduce the whole landscape of conformations that Glut1 can take in order to elucidate the details of the mechanisms of Glut1 transport

To do so, a mechanistic model of the protein was built to obtain the behavior of the whole molecule. The glucose transfer doesn't seem to depend on the global conformational change of the transporter. The residues which interact with glucose the most are the same residues identified to reduce glucose activity or those associated with Glut1 Deficiency.

Role of Glut1 in Endothelial Cells

Prof. Dr. Katrien De Bock

Endothelial cells are highly glycolytic and generate the majority of their energy via the glycolytic breakdown of glucose to lactate - glucose derived energy is required for optimal endothelial cell function.

We were interested in understanding how Glut1 contributes to glucose transport and glycolysis. When Glut1 was inhibited through pharmacological inhibitors, glucose transport was completely inhibited and endothelial cell glycolysis was significantly reduced.

Deletion of endothelial Glut1 led to impaired outgrowth in the developing retinal vasculature. Endothelial cells rely on Glut1 to take up glucose and to fuel their own metabolism during vessel growth.

Glucose transport over the BBB is Glut1 dependent. It's still to be determined whether Glut1 is involved in transport to other organs such as skeletal muscle. This process is tightly controlled by insulin. Muscle is the main site of glucose disposal. Loss of endothelial Glut1 leads to reduced muscle glucose uptake. Endothelial Glut1 is required for glucose uptake in muscle.

Loss of endothelial Glut1:

- impairs insulin signaling in muscle
- activates a pro-inflammatory response
- leads to accumulation of macrophages

Macrophage proliferation is instructed by endothelial cells. Depletion of Glut1 from muscle endothelial cells leads to the activation of an inflammatory angiocrine signature, which causes local macrophage proliferation/activation and muscle insulin resistance. The role of Glut1 in endothelial cells goes beyond the regulation of glucose transport.

Glut1 Regulation in Astrocytes

Prof. L. Felipe Barros

In regards to activation of glucose during events such as a cognitive task, the concentration gradient between the plasma and parenchyma is almost maximal. However much the concentration of glucose is reduced, this is not enough to cater for the increased flux.

Lactate concentration in the extracellular space of the brain goes up before the glucose concentration goes down. Almost immediately after local activation of metabolism produces an increase in extracellular lactate.

It is thought that neuronal activity communicates with the astrocyte through an increase in extracellular potassium; potassium is very important in the first seconds to minutes. Potassium stimulates astrocytic Glut1 and lactate is potassium dependent.

This complex network of signaling events requires glucose to be sustained and this is done by a fast activation by the glucose transport of astrocytes which provides cells with more lactate, oxygen, and glucose.

At the Blood Brain Barrier

Modeling Glut1 Deficiency in a Dish: Lessons from stem cell-based models of the human blood brain barrier

Abraham Al-Ahmad, PhD

Mission: to model understand and treat neurodegenerative disease by focusing on neurovascular function

Current challenges in modeling the blood brain barrier (BBB):

- The use of lab animals to study the transport of glucose across the BBB provides only limited information and requires invasive procedures.
- The use of in vitro cells to study the transport of glucose across the BBB can provide a detailed understanding on how glucose can cross the BBB but suffer from the lack of barrier function, or species differences.
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Generated Glut1 Deficiency human induced pluripotent stem cells (iPSCs)
SLC2A1+/- iPSCs appear not affected by Glut1 knockdown
Conclusions:

- iPSC-derived brain microvascular endothelial cells are capable of displaying Gluts expression similarly to somatic cells
- Because of their ability to form tight barriers, these iPSC-derived brain microvasculature endothelial cells (BMECs) are more suited to model the human BBB in vitro when it comes to assessing the glucose transport
- iPSC-derived BMECs also show a glucose metabolism and high reliance to glucose
- SLC2A1 knockdown cells exhibit major changes as undifferentiated stem cells but show detrimental effects on the barrier function and Glut1 expression, impacting the glucose uptake compared to the control line.

High Throughput CRISPR Screening to Identify Regulators of Glut1 Expression

Ethan Lippmann, PhD

The Lipmann lab mission is to model, understand and treat neurodegenerative disease by focusing on neurovascular function.

Strategy: combine biomolecular and biomedical engineering principles with molecular biology analyses for comprehensive in vitro and in vivo studies.

Caco-2 epithelium is the model cell line used in the Lipmann lab because they have high levels of Glut1 expression.

They have knocked out Glut1 from Caco-2 repeatedly in other studies which has validated this.

Although the majority of gene knockouts lead to loss of Glut1 expression, there are a subset of knocked out genes that lead to increases of Glut1 expression .

Blood Brain Barrier on-a-chip: Nanomedicine for Glut1 Deficiency

Angels Garcia Cazorla, MD, PhD

Lab On-Chip Devices integrate one or several laboratory functions on a single chip form.

The purpose of the chip is to detect the presence of a particular virus. The aim is to mimic the mechanical, biochemical, and physiological properties of the brain. There are various types of Lab On-Chip models and there is a plan to develop a blood brain barrier on a chip for Glut1 Deficiency in collaboration with Institut de bioenginyeria de Catalunya and Hospital Sant Joan de Déu.

In order to build up this BBB on a chip the cell type, the materials for the chip extracellular matrix, the microenvironment that will be used to mimic the physiological condition need to be considered.

This project is focused on the development of a 3D barrier interface to mimic in vitro the physiological behavior of the BBB and study the flow of the metabolites and the pharmacology efficiency of drugs for Glut1 Deficiency.

Glut1 The Transporter: in Disease

Glucose Transporters in Brain During Health, Diabetes, and Stroke

Prof. Hermann Koepsell

Glucose transporters expressed in brain belong to the GLUT and SGLT families

- in rodents and humans, cerebral expression of Glut1 1-4 was observed
- the cerebral localization of Glut1 and Glut3 was determined in humans and rodents
- the cerebral localization of Glut2, Glut4, and Sglt1 and Glut3 was determined only in rodents
- functions of glucose transporters in brain were only investigated in rodents

The GLUTs are facilitative diffusion systems of glucose across the plasma membrane. Plasma membrane abundance of Glut4 is increased by insulin.

Glucose transporters supply neurons with D-glucose. Increased neuronal firing in response to learning and exercise upregulates expression of Glut1-4 in brain. Blood glucose is regulated in hypothalamus involving glucose sensing via glucose transporters.

In humans, type 1 diabetes (T1D) has been identified as a risk factor for development of cognitive impairment. In humans, correlations between type 2 diabetes (T2D) and deficits in memory functions were observed.

Stroke leads to decreased glucose concentration in nerve cells, decreased ATP, blockage of sodium potassium ATPase which leads to a decrease in membrane potential and to an increase of intracellular calcium, the stimulation of excitatory receptors.

Upregulation of Glut1 in microvessels and astrocytes after brain ischemia may represent a protective mechanism increasing glucose and oxygen supply similar to the increase of microvessels.

Conclusions:

- Glut1 plays a pivotal role for the transport of D-glucose across the BBB and across membranes of astrocytes.
- D-glucose uptake in neurons is usually mediated by Glut3, in hippocampal neurons it may be also mediated by Glut 2 and Glut 4
- An increased energy demand during neuronal activation is compensated by upregulation of glucose transporters in BBB, astrocytes and neurons

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Lessons from Cancer

Etienne Meylan, PhD

Are tumor cells using glucose differently than healthy cells?
In tumor cells, even in the presence of oxygen, glucose will be converted into lactate through glycolysis which is much less able to generate ATP similar to healthy cells without oxygen.

What happens to Glut1 in cancer?

- Cancer cells take up glucose more efficiently; there is a gain of function of glucose transporters (particularly Glut1 and Glut3)
- High Glut1 expression in tumors is associated with poor prognosis

Is Glut1 functionally important for tumor growth and expression?

- When comparing tumor progression in the presence or absence of Glut1, not many things changed.
- Tumor growth, weight, and number were not significantly different.
- Another Glut may be compensating for the loss of glut1 in tumor cells – Glut3

With a double deletion of Glut1 and Glut3, tumor number was reduced, tumor growth was strongly impaired, the weight was reduced and the survival of mice was increased. Therefore, both Glut1 and Glut3 are involved in tumor growth.

Lessons from Alzheimer's Disease

Berislav Zlokovic, MD, PhD

The AD field is dominated by work in the amyloid beta area, but other factors may be involved and should be explored for disease pathogenesis and possible treatments.

The blood brain barrier is dysfunctional in many neurodegenerative diseases. The link between BBB dysfunction and

neurological diseases is best illustrated by human rare monogenic neurological diseases. There are 20 or so of these diseases.

The BBB plays the role of a metabolic organ in the brain, an ecosystem of the brain and provides everything the brain needs for its metabolism. An example is Glut1, which is present in 30 million copies in the brain and BBB and supplies brain with glucose.

Impaired BBB function leads to neuronal, synaptic, and CNS dysfunction. BBB breakdown is an early biomarker in human cognitive dysfunction. AD models show Glut1 deficiency in blood vessels, much less Glut1 in entire brain and in microvessels.

Glut1 deficiency in AD APP mice accelerates BBB breakdown and works with amyloid beta to accelerate neuronal dysfunction as well as development of microcephaly. There is progressive increase of BBB breakdown with loss of endothelial tight junctions when there is Glut1 Deficiency in AD mice. Neuronal dysfunction, neuron loss, and microcephaly follow metabolic and vascular changes. There is a huge accumulation of amyloid in AD mice that are Glut1 deficient.

New work needs to be done in animal and human models to determine how Glut1 deficiency contributes to the neurodegenerative process and cognitive impairment in AD. We need to determine whether Glut1 is a viable therapeutic opportunity. If there is comparable function for Glut1 in AD and Glut1 Deficiency syndrome, it could represent a paradigm shift in disease pathogenesis and treatment.

Brain Glucose Metabolism:

Overview of Brain Metabolism and Connections Excitability
Gary Yellen, PhD

What does the brain need energy for?

- Ion gradients for electrical signaling which functions as a battery
- Synaptic signaling where one neuron connects to another. At those connections there are small vesicles containing neurotransmitter molecules. thos vesicles release their contents into the synaptic cleft between the two cells which creates electrical signals in the postsynaptic and receiving neuron.
- This requires energy dependent organization, requires a calcium gradient, vesicles need to be recycled and all of this takes energy.

What happens when the neurons don't have enough energy?

- These signaling elements begin to fail
- This imbalance in normal signaling can produce seizures and damage to cells

The brain gets this energy from fuel molecules that come from the food you eat such as glucose which can generate ATP for energy. For Glut1 Deficiency the brain can fortunately use another form of energy, ketones.

Just making those ketone fuels available, you inhibit glycolysis, so you switch the balance from using glucose to using ketones – this is done to prevent an energy crisis.
The tradeoff in cellular fuel utilization of increased ketone body utilization and decreased glucose utilization leads to changes in brain excitability / susceptibility to seizures.

Central Nervous System Fuels

Mary McKenna, PhD

Fuel use in the brain changes during development

- There is a transition from high ketone use to reliance on glucose as primary fuel entering brain
- Within the brain other fuels can be used by brain cells (ketones, lactate, glutamate, glutamine, fatty acids)
- Brain cells have preferred fuels but they are metabolically flexible and can utilize many fuels

Substrate use in brain is limited by:

- Uptake across BBB and transport into cells
- Glut1 is the glucose transporter used by endothelial cells of BBB, astrocytes and oligodendrocytes
- activity and presence of enzymes needed to metabolize the fuel/substrate

Substrate use is also influenced by:

- metabolite concentration and fluctuations
- post translational modification of proteins
- cellular damage

Functional activity in developing brain parallels the increase in glucose metabolism. Substrates alone are not enough- many vitamins and minerals are needed for metabolism of substrates, and many minerals are required coenzymes needed for enzyme function.

Brain development is a highly regulated process that requires a lot of fuel for energy and biosynthesis of nucleotides, proteins, and lipids. Myelination is needed for normal nerve conductance and it begins prenatally.

Metabolism of fuels is crucial to provide energy for all cellular processes in brain and all developmental processes. Most energy use is related to signaling. Brain metabolism is compartmentalized- different types of cells have different enzymes and transporters. It's important to note that glucose has some roles that cannot be filled by other substrates.

None of the other fuel substrates can achieve all of the things glucose can accomplish. For example, ketones enter at the TCA cycle and can be used for energy, but not the other things that glucose can achieve. It's important to ask whether the diets contain all other necessary nutrients for energy metabolism.

A Cerebral Lymphatic System- Potential Effects on Energy Metabolism

Maiken Nedergaard, PhD, DMSc

The Glia-Lymphatic System (or glymphatic system) is a network of vessels that clear waste from the central nervous system. It is a fluid transport pathway into the brain. It pulses CSF into the brain and relies on the periarterial and periventricular spaces to infuse the brain and then remove wastes, mostly during sleep.

Hexokinase expression is correlated with increased glucose requirement and it is found that neurons are consuming much more glucose than astrocytes. The conclusion is that the glia-lymphatic system can transport glucose, and this could be a new target in diseases that require passage through BBB because you could bypass the BBB deficits and deliver glucose in a way very similar to the BBB and it would be utilized by cells in the same way.

Glycogen and Lessons from Lafora Disease

Matt Gentry, PhD

Lafora disease is a fatal glycogen storage disease in childhood dementia. Lafora Disease patients develop normally in the first 10 years of life and then experience an epileptic episode. They begin to undergo a rapid dementia around 17 or 18 years. Sugar like aggregates, referred to as Lafora bodies, are what drive the disease.

Mouse models have been generated to replicate the disease as well as an antibody to be able to examine the Lafora bodies in the brain. The goal is to eliminate the Lafora bodies when they form or to stop the production from the beginning. In Lafora Disease, they can synthesize glucose but there is an accumulation of glycogen. They can make glycogen but not release that glucose.

Using genetic models, it has been shown that if you decrease or stop the rate of synthesis of glycogen of the brain, you rescue neurodegeneration, epilepsy and the neuroinflammation that's seen.

Antibody enzyme fusions are often used in cancer settings where an antibody targets a specific cancer cell type and delivers a cytotoxic drug to that cell type.

A company has another approach to this where they have identified an antibody fragment that can gain access into the cell cytoplasm and the nucleus. This was applied to Lafora Disease by identifying an enzyme that can degrade Lafora bodies and fused that to the fragment which showed that this fusion could degrade Lafora bodies.
This was then brought to the mouse model. Animals treated with the drug cleared the Lafora bodies and this treatment was also able to rescue glycosylation.

Role of the Astrocyte Neuron Lactate Shuttle in Glut1 Deficiency

Pierre Magistretti, MD, PhD

We are interested in understanding the cellular and molecular mechanisms that underlie the coupling of synaptic activity with metabolic responses.

Astrocytes are involved in metabolic and energetic support as well as neuronal plasticity. Astrocytes are key players to deliver energy into neurons. Glucose is mainly converted to lactate by astrocytes for the use of neurons.

We identified molecules that increase activity of Glut1 in astrocytes, increase glucose levels in the brain, and increase glucose utilization by the brain.

Use of a drug that targets astrocytes to increase lactate in Glut1 Deficiency mice proved to increase glucose and lactate levels in the brain after oral administration.

Ketogenic Diets:

Introduction and Session Moderation

Jong Rho, MD

Ketogenic diets are the treatment of choice for Glut1 Deficiency, although not all respond favorably at every age. The following presentations help highlight where we've come as a field of study.

Evolution of Ketogenic Diets Over the Past Quarter Century

Elizabeth Thiele, MD, PhD

The idea of using the ketogenic diet to treat epilepsy dates back to a thousand years ago.

There are now 4 diets being used around the world for the treatment of refractory epilepsy

- Classic Ketogenic Diet (KDT)
- Medium Chain Triglyceride Diet
- Modified Atkins Diet (MAD)
- Low Glycemic Index Treatment

Last year, there was a randomized control study published comparing KDT, MAD, and Low Glycemic. Results showed that there was not a significant difference in efficacy.

Today we are seeing:

- increasing utilization of dietary therapy in adults with epilepsy
- increasing use in the treatment of non-refractory epilepsy and early implementation
- increasing interest in the role in other medical conditions including other neurological disorders in cancer
- increasing interest in mechanisms of action and what that can tell us about epilepsy and metabolism
- increasing availability of commercial products
- increasing KDT community including strong advocacy groups
- global consensus clinical guideline for implementation and management of dietary therapy

Dietary therapy remains the most effective treatment for refractory epilepsy even with the development of many antiseizure medications

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Is the Efficacy of Ketogenic Diet in Glut1 DS Strictly Due to Ketone Body Utilization

Karin Borges, PhD

The expected effects of an MCT ketogenic diet are:

- reduced ATP production
- reduced formation of lipids and amino acids
- reduced glutamine and GABA production

Medium Chain Triglycerides (MCT) are easily turned to ketone bodies, especially when glucose is low.

The expected effects of an MCT ketogenic diet are ketone production but is it possible that octanate and decanate are directly affecting acetyl CoA production.

When people take MCTs in small doses, in addition to a regular diet, they will have steady plasma levels of octanoic and decanoic acid. They will also produce ketones.

Octanoic acid is mostly metabolized by astrocytes in acute mouse brain slices
When you add on MCT oil in dogs with epilepsy, 50% have seizure reduction.

It is very difficult to distinguish direct effects of medium chain fatty acids (MCFA) vs those on ketone bodies. MCFA enter the brain and are expected to contribute energy and carbons for amino acids and lipids. Medium chain fatty acids and MCT seem to be anti-convulsive themselves but definitely need to be explored further.

Neuroprotective Activity of the Ketogenic Diet on Cognition and Behavior

Robin SB Williams, PhD

Neuroprotection involves the ability for a therapy to prevent neuronal cell death by inhibiting the pathogenic cascade that results in cell dysfunction and eventual death. This is a process intimately related to epileptogenesis and seizure propensity and a demonstrated mechanism of the KDT.

Neuroprotective effects of the classical KDT have been proven in rodent models.
Direct neuroprotective effects of ketones in vitro, cell death and damage is greatly reduced.

Direct neuroprotective effects of MCT ketogenic fatty acids improved rodent status epilepticus.

Mitochondria provide energy for the cell and it is thought that during a seizure, the hyperactivation of neurons leads to a strong reduction of energy stores which induces cell death.

Both classical and MCT diets elevate mitochondria number, protecting against this death. Multiple mechanisms have been proposed:

- Mitochondrial regulation of energy levels
- Reducing the excitatory toxic effect of enhanced neurotransmitter signaling
- Signaling pathways known to be neuroprotective

The Effects of the Ketogenic Diet on Cognition and Behavior

Susan Masino, PhD

In a case report done in the US, a patient with Glut1 Deficiency showed significant improvement in behavior, sleep, and gross motor functions subsequent to initiation of KDT. In another paper, 4 Glut1 Deficiency patients experience remarkable improvements in movement disorder and cognitive function with the Modified Atkins Diet.

Locomotor activity of mice on the ketogenic diet decreased initially but then normalized or even improved over time.

Feeding fruit flies beta hydroxybutyrate found no change in locomotor activity with or without a traumatic injury.

There's a lot of improvement in mice with autism who are on the diet. However, there was a significant decrease in aggression in animals fed the ketone supplement. In looking at the KDT in autism mouse models, there are a lot of improvements across mouse strains and there were no negative effects on cognition or locomotor activity with KDT. Male autism mice on KDT were much more social.

Case studies on community-based dogs with epilepsy that ate KDT had seizure as well as behavioral improvements.

Future Directions:

- Systemic approach to try to identify knowledge gaps (risks)
- Research on males and females, range of species
- Consider dual opportunities - modify disease and promote health
- Opportunities for efficacy and "dosing" with ketone supplements
- Focus on metabolic component of neurological disorders
- Identify predictive opportunities/timeline to increase access, uptake
- Translate unbiased research for the public and into public policy

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Roundtable Discussion Summary:

Glut1 Deficiency Clinical Roundtable:

- There are quite different clinical presentations between children and adults and we need to understand how we can address that and what are the mechanisms behind that
- Treatment beyond KDT; it works in most patient but not all, so what can be done to help them
- Link between bench to bedside is something parents and doctors need to know
- It was very obvious that things that affect quality of life such as speech and emotional problems are aspects that are very troublesome for patients and families, and we have to find novel ways to deal with that

Glut1 Deficiency Research Roundtable:

- What is the right cell model for screening for drug discovery?
- Now we're looking at activators for Glut1 but there may be a better model
- What are the endpoints for the therapeutics that are being developed?
- Better characterization of Glut1 Deficiency - single cell sequencing, autopsy characterization

Gene Therapy Roundtable:

- There are key questions that remain to be answered regarding gene therapy
- Some of these gaps include: AAV9 - is this an optimal capsule for inducing Glut1 endothelial cells
- What's the minimum amount of Glut1 needed to prevent disease or for optimal therapeutic outcome?
- What is the optimal route of administration and is it possible to expand the therapeutic window of opportunity?
- Development of a larger model, such as pig

Glut1 – The Transporter Roundtable:

- Big gap in the understanding of the transporter and its regulation
- What are the transcriptional and post transcriptional level regulators?
- What determines trafficking, expression, function, configuration?

Blood Brain Barrier Roundtable:

- The different mutations could influence different properties of Glut1 and the blood brain barrier
- The genetics might be individually regulating how the transporter is behaving
- How to maximize transport of glucose through the BBB
- Modulation of transport to the BBB

Glucose Metabolism:

- Technique is important in studying the and analyzing the preclinical brain
- Substrate competition versus utilization
- Substrate driven toxicity
- How does glycogen fit into the Glut1 mix?

Ketogenic Diets Roundtable:

- Critical gaps: when the diet doesn't work, why does that happen?
- Is it a question of biomarkers, genotypic or phenotypic variances
- When it doesn't work, what do we do next?
- Is continuous glucose monitoring something we should be implementing?
- Intracerebral ketone and glucose levels
- Newborn screening – would we be able to intervene earlier?
- Comparison of diets among different groups

Patients and Families:

- Patient stories help educate the field
- Good feedback from the collaborative drive going forward
- New uses of existing treatment
- Beneficial to have a good set of doctors who listen and continue learning
- Importance of having pediatricians aware of this disease for early diagnosis

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Poster Session:

Find listing, abstracts, posters, and presentations on the Glut1 Deficiency Foundation website